CORN STEEP LIQUOR IN MICROBIOLOGY

R. WINSTON LIGGETT[1] AND H. KOFFLER

A. E. Staley Manufacturing Company, Decatur, Illinois, and Purdue University, Lafayette, Indiana

The publicity given to the development of the penicillin industry also has called attention to the value of corn steep liquor as a source of nutrients for microorganisms. Although considerable information on the properties of corn steep liquor has been accumulated, attempts to integrate this information have been rare (cf. 38). An effort will therefore be made in this review to describe the production and properties of corn steep liquor, and to evaluate its usefulness in microbiology.

Production of corn steep liquor

Since corn steep liquor is a by-product of the corn wet-milling industry it would be insufficient to discuss its manufacture apart from the whole process in which corn, after having been shelled and air-cleaned, is soaked, and then fractionated into its principal components by a combination of flotation and wet-screening procedures.

To avoid losses of raw material and to keep sewage disposal problems to a minimum, practically complete recovery of the solids is desired. This is accomplished by the so-called "bottled-up" process whereby water is reused in a counter-current flow with respect to the corn and losses of the solids are kept to less than 0.5% of the dry substance of the corn. The technology of this process is discussed in detail by Kerr (26). A popularized but authentic description can also be found in a publication by the Corn Industries Research Foundation (7). For a discussion of the water balance and sewage disposal problems see Greenfield, Cornell, and Hatfield (20).

The corn is first soaked, or steeped in open wooden tanks at 45 to 52 C for 40 to 48 hours. Five to seven gallons of water are required for every bushel of corn. The water used in steeping is process water that has been used previously in other phases of the process, for example, the overflow from the gluten settling tank. During steeping the soluble materials are dissolved, the corn is softened, and its structure weakened and broken, which facilitates the grinding and further separations of its components. Just before the process water enters the tanks, SO_2 is added to prevent putrefaction and to assist in the extraction of the soluble compounds. The concentration of SO_2 is initially from 0.1 to 0.2%, but since most of the SO_2 is absorbed by the corn, it is lowered to 0.05% five hours after addition, and to 0.01% within ten hours. Moving in a general counter-current fashion, the most dilute water is placed on corn that has been steeped the longest and is transferred continuously in the direction of the corn most recently introduced. In this manner, the steep water having the highest concentration of

[1] Present address: American Sugar Refining Company, Philadelphia.

solutes is used on corn just entering the system after which procedure the water is withdrawn and concentrated to a solid content of approximately 50%. This concentrate, crude corn steep liquor, may then either be combined with gluten and fibrous materials and sold as animal feed, or used for microbiological purposes, with or without further processing.

From the steeps the soft corn is transferred to the mill house where the multistage milling and the separation of the components of corn take place. After a coarse grind in an attrition mill, which consists of two large metal discs with metal teeth, the slurry is suspended in water and slowly passed through the germ separators. In these large troughs the germs, rich in oil, float to the top and are thus separated from the heavier materials which settle to the bottom. This residue, which consists of starch, gluten, and hull, is transferred to the reels, rotating coarse sieves of stainless steel, where the hulls are retained. To insure complete recovery of the valuable germs, the hulls are reground and passed again through the separators. The germs are moved over a battery of reels, washed free of adhering starch, and dehydrated, and the corn oil is extracted. The degerminated corn is partially freed of water in separator reels, and transferred to the Buhr mill which consists of two large granite stones, one on top of the other. The water removed at the separator reels contains considerable quantities of starch and gluten, which after being rid of coarse particles pass on directly to the table house. The degerminated corn is then reground in the Buhr mill, to a fineness sufficient to allow the separation of starch and gluten without at the same time also grinding the hulls and thus causing contamination of the starch with fibrous materials. Rotating reels are used to sieve out the coarse fiber, and rapidly moving silk shakers to remove the last pieces of fiber.

The mixture of starch and gluten is transferred onto the starch tables, which are flat-bottomed troughs, approximately 120 feet long, and slightly tilted so that the suspension will flow slowly towards the far end of the trough. The starch particles, being heavier than gluten, are deposited on the tables while the gluten flows off at the end of the table. The starch is washed off the tables, dewatered again by filtering on rotary filters, and finally dried. Washing and filtering may be repeated several times to remove all soluble products from the starch. Centrifuges are coming into vogue now and may eventually replace "tabling" as a means of separating the starch from the gluten. The gluten is recovered by allowing the liquor to stand in large tanks, the so-called gluten settlers, until it settles, and by filtering and drying the settled material. Figure 1 diagrams the various steps of the process. It is important to remember that large volumes of water are used, approximately 30 to 50 gallons per bushel of corn, and that much of this water is recovered from the filters and settling tanks and recycled in the process. Ideally, fresh water is introduced only for the final washing of the separated starch, and withdrawn only to the steeps, but such a multistage process is not easily kept in complete water balance (Cf. 20). Irregularities in procedure occur often, changing the character of the corn steep liquor.

During the steeping period and during the other phases of the process, there occurs an active natural fermentation, essentially lactic in nature. In spite of

the low pH, 3.8 to 4.5, the total viable count of organisms frequently runs into billions per ml. Variations of the order given in table 1 have been noted. In

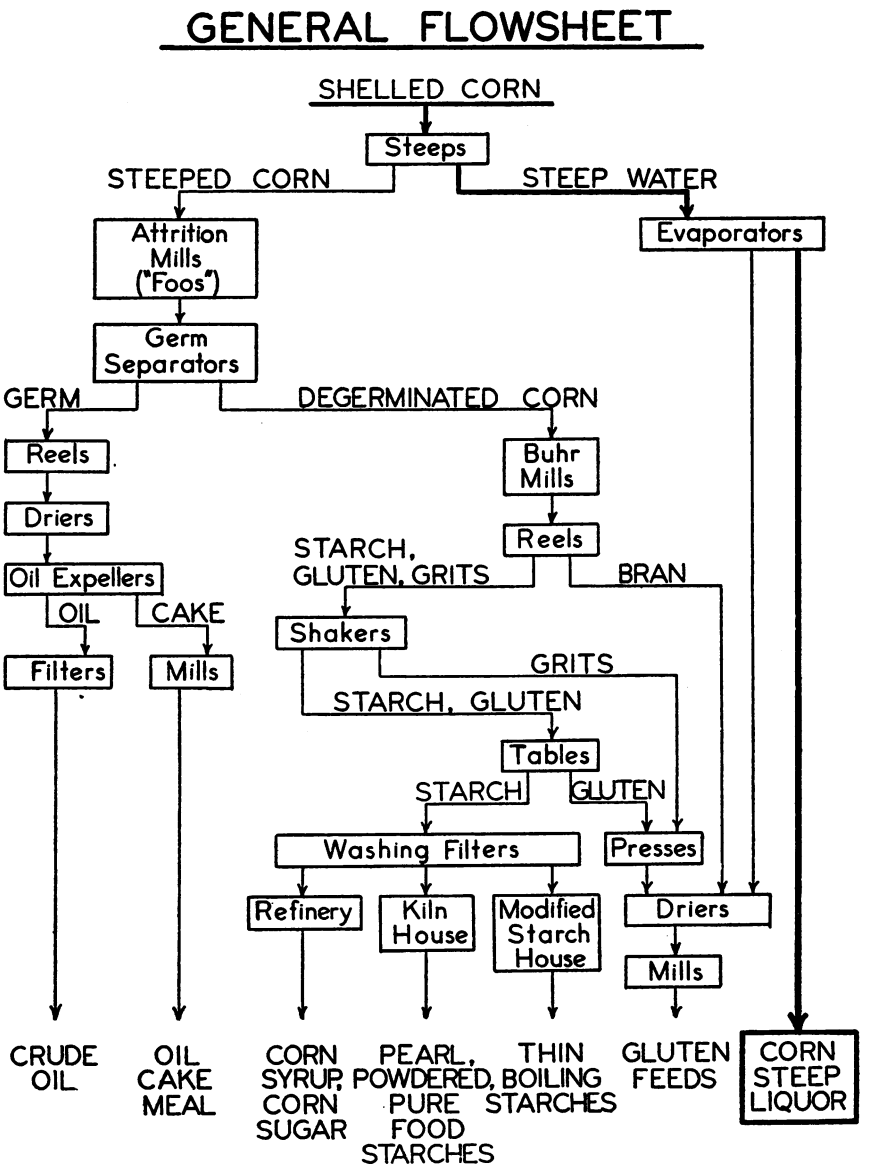

FIG. 1. GENERAL FLOWSHEET OF THE CORN WET-MILLING PROCESS

The flow of water is counter-current to the flow of materials as shown in the diagram. To maintain the water balance in the plant, water may be in process and storage for as long as two weeks. The water in the evaporator may come from any stage of the process.

general, the lowest microbial counts are found in process water containing the highest amount of SO_2, while the highest counts are found in the oldest process

water which is used on the most recent corn. In addition to the thermophilic bacteria, which are nearly all rods, other types have also been isolated by incubation at lower temperatures, including a variety of cocci. The predominant organisms, however, are thermophilic lactics, including both spore-formers allied to the "flat-sours", and representatives of the genus *Lactobacillus*. It should be pointed out that the lactic acid fermentation is not only important in the manufacture of starch but also in that of corn steep liquor of a quality desirable for microbiological purposes. The corn proteins are relatively water-insoluble, but they do swell and dissolve to some extent in acidified water. A certain amount of acidity is therefore essential for the efficient separation of starch from gluten, and the lactic acid complements the action of SO_2. The solution of the corn proteins, however is not entirely chemical, but also involves bacterial enzymes,

TABLE 1

Microörganisms in corn steep water

TYPE	TOTAL COUNT
	numbers/ml
Aerobic bacteria[a]	30,000–1,000,000
Anaerobic bacteria[b]	5,000–20,000
Microaerophilic bacteria[c]	10,000,000–10,000,000,000
Yeasts[d]	10–1,000,000

[a] Surface colonies on brom cresol purple-dextrose agar plates, at 45 C. Organisms of *Bacillus subtilis* and *Proteus vulgaris* type; flat-sours not counted.

[b] Colonies in Brewer's thioglycollate agar tubes, at 45 C.

[c] Colonies in tryptone-yeast extract-dextrose agar tubes, at 45 C; primarily lactics. A count as high as ten billions occurs only in steeping systems that are out of balance; a count of one billion is more common as the upper limit.

[d] Colonies on wort agar plates, at 30 C.

which catalyze the breakdown of the native proteins to smaller units. Owing to this bacterial activity not only can starch be more easily separated from gluten, but the resulting corn steep liquor is high in amino acids and polypeptides, which are excellent sources of nitrogen for almost all microörganisms. If the bacterial fermentation is to accomplish such purposes it must be regulated, at least to a certain extent, by the control of certain environmental factors. For example, the relatively high steeping temperature, in the presence of readily available carbohydrates, favors the lactic acid fermentation and thus keeps putrefaction and alcohol formation to a minimum.

Essentially then, corn steep liquor consists of concentrated corn solubles, which have been extracted during the steeping process at approximately pH 4 and at a temperature of 45 to 52 C in the presence of SO_2 and an active lactic acid fermentation. In addition, it includes soluble materials collected at other stages of the milling or separation processes; since process water may be retained in the plant for approximately two weeks it is not surprising that additional fermentations occur at many points. The yeasts isolated from the steep water do not

have the morphology and cultural characteristics typical of organisms cultured for several transfers on wort agar media, and apparently do not multiply actively.[2] However, at other stages of the process, which are conducted at lower temperatures, normal yeast development is thought to occur. For example, in the gluten settling tank and thickeners, where temperatures may go as low as 30 C, a scum of film yeasts occurs. Killinger (28) has emphasized the prevalence of yeasts and yeast-like organisms in starch process water.

The degree of fermentation that the solids of corn steep liquor have undergone varies greatly from plant to plant, and considerably even in one plant from time to time. To a certain extent, this variation can be controlled,[3] but there is also an apparent seasonal variation in the microbial flora, which so far has resisted control.

Chemical composition

The main disadvantage of corn steep liquor in microbiology is its variable composition. This variability may depend somewhat upon the type and condition of the corn but even more upon a multitude of variables involved in the processing of starch. On the other hand, corn steep liquor is an inexpensive alternative (4 to 6 cents per pound of liquor) to much more expensive materials, such as yeast extract and peptone.

Corn steep liquor has a pH of 3.7 to 4.1, a specific gravity of 1.25, and on proximate analysis a general composition as indicated in table 2. On the average 6.9% of the corn solids and 30% of the corn nitrogen are found in the steep liquor. Ninety-five per cent of all samples have a nitrogen content of 3.85 to 4.1% while only 5% contain nitrogen below or above these limits. Similarly the majority of samples contain 1.45 to 1.65% amino nitrogen and 0.15 to 0.30% volatile nitrogen, largely ammonia. The amino N/total Kjeldahl N ratio for 95% of all samples is from 0.38 to 0.40%, indicating that the nitrogenous compounds present are, to a large extent, amino acids and polypeptides. Low amino N/total Kjeldahl N ratios are encountered in samples that have not undergone a vigorous lactic acid fermentation. To illustrate: the lot giving the lowest ratio ever encountered in these laboratories, also showed 11% of reducing sugar (determined as glucose), 5.1% of lactic acid, 0.15% of volatile nitrogen, and 0.1% of volatile acid (determined as acetic acid). This indicates a steeping system badly out of balance and one likely to be accompanied by the formation of scaly deposits in the evaporators. This scale or precipitate, spoken of as

[2] On first isolation on wort agar the yeasts from the steeps often give pin-point colonies consisting of very small, coccus-like cells. On the second or repeated transfers the organisms attain normal size and shape. *Torulopsis* shows normal morphology and colonial development even on first isolation. Among the yeast genera present, as kindly identified by Dr. L. J. Wickerham of the Northern Regional Research Laboratories, were *Trichosporon*, *Torulopsis*, and *Mycoderma*.

[3] An interesting example of this is a patent by Kerr and Berlin (27) which claims a reduction of scaling in evaporators if a secondary fermentation is encouraged by addition of carbohydrates and aeration.

"liver", consists almost entirely of coagulated proteins and insoluble salts of calcium and magnesium. Under normal steeping practices the coagulable proteins are degraded to smaller fragments, the greater number of which are no longer heat coagulable, and the insoluble salts are kept in solution by the lactic acid. Highly fermented steep liquors, on the other hand, show not only a high amino N/total Kjeldahl N ratio (0.50) but also a low sugar content (less than 1 to 2%), a high concentration of lactic acid (13 to 15%), and higher concentrations of volatile nitrogen compounds (0.40%) and volatile acids (0.3%) than poorly fermented steep liquors.

According to Cardinal and Hedrick (5), over 95 per cent of the total nitrogen in steep liquor is accounted for, after hydrolysis, by ammonia and the following amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, and valine. More than one-quarter of the nitrogen is present as alanine.

TABLE 2

General analysis of corn steep liquor

DETERMINATION	PER CENT	DETERMINATION	PER CENT
Water[a]	45 -55	Lactic acid[a]	5 -15
Total Kjeldahl-N[a]*	2.7 - 4.5	Ash[a]	9 -10
Van Slyke Amino-N[b]*	1.0 - 1.8	Volatile acids[d] (as acetic acid)	0.1 - 0.3
Volatile-N[c]	0.15- 0.40	SO_2[d]	0.009- 0.015
Free reducing sugar (as glucose)[a]	0.1 -11.0		

[a] Based on determinations made on 1000 different lots.
[b] " " " " " 50 " "
[c] " " " " " 15 " "
[d] " " " " " 10 " "
* The Amino-N/Total Kjeldahl-N ratio ranges from 0.30 to 0.50.

Corn steep liquor contains considerable amounts of the B-complex vitamins, with the exception of thiamine, which is usually low or absent, probably because it is destroyed by the SO_2 treatment during steeping. Tanner, Pfeiffer, and Van Lanen (59) analyzed a corn steep liquor medium used in the penicillin industry and found the following vitamin content, recalculated on the basis of micrograms of vitamin/gm of wet corn steep liquor: riboflavin, 5; niacin, 819; pantothenic acid, 23.8; pyridoxine, 19.1; and biotin, 0.125. Their data agree closely with our values: vitamins A, D, E, K, C and thiamine, usually 0; riboflavin, 5 to 10; pyridoxine, 25 to 400. The inositol content is uniformly constant and at least 1 mg/gm of steep liquor. Approximately half of the inositol occurs as phytin.

Table 3 presents information on the composition of corn steep liquor ash. Some of the discrepancies between the values listed may be due to the use of different analytical procedures rather than to actual differences in composition. Obviously the ash of corn steep liquor contains a wealth of mineral nutrients;

TABLE 3

Composition of the ash of corn steep liquor

ELEMENT	I % OF DRY MATTER	II % OF DRY MATTER
Al		0.032
Ca	0.5 -1.5	
Cd		0.0029
Cu	0 -0.001	0.0033
Fe	0.01 -0.05	0.052
Pb		0.055
Mg	0.5 -1.0	1.05
Mn	0.004	0.012
Mo		0.0006
P	2.0-3.0	2.75
K	1.0-2.0	
S	0.34	
Zn	0.005	0.0005

I = range of quantitative determinations made on 10 different lots.

II = data on a single lot; from Perlman (48).

A semi-quantitative spectroscopic analysis on a single lot, given by Koffler, Knight, and Frazier (33) indicates the presence of the following elements: Al, As, B, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Ni, P, K, Si, Ag, Sn, W, Zn. The following metals were not detected: Sb, Be, Bi, Cd, Cb, Ce, Au, La, Hg, Pt, Sr, Ta, Ti, V, and Zr. Another spectroscopic analysis, also on a single lot, is presented by Cook, Tulloch, Brown, and Brodie (16). These workers found the presence of the following elements: Ba, Pb, Mo, Ni, Rb, Sn, V, and Zn; the following were absent: Cr, Co, Li, and Ag.

TABLE 4

Some uses of corn steep liquor in microbiology

USED IN THE PRODUCTION OF
Yeast food (1)
Yeast (62)
Leavened dough-products (64)
Yeast stimulant (63)
Organic acids from cellulosic material (35)
Beer (18)
Bread dough (49)
Brewing adjunct (65)
Sorbose by *Acetobacter suboxydans* (66)
Ketogluconic acids by *Acetobacter suboxydans* and an unnamed organism (58)
Gluconic acid by *Aspergillus niger* (47)
Itaconic acid by *Aspergillus terreus* (40, 41, 42, 43)
Penicillin by *Penicillium notatum-chrysogenum* (44, 45)
Pentonic acids by *Pseudomonas* (39)
Riboflavin by *Ashbya gossypii* (60)
Subtilin by *Bacillus subtilis* (37)
Amylase by *Aspergillus niger* (36)

especially calcium, iron, magnesium, phosphorus, and potassium occur in high concentrations.

Use in Nutrient Media

Although Behr (1), who is credited with the invention of much of the modern wet milling process, suggested corn steep liquor as a nutrient for microörganisms as early as 1909, its use in microbiology until recently was limited to yeast fermentations. However, workers at the Fermentation Laboratories of the United States Department of Agirculture and others have advocated the use of corn steep liquor in other fermentations so enthusiastically that its usefulness as a general substrate for microörganisms is being recognized. It hardly needs to be mentioned that corn steep liquor has contributed, in a large measure, to the rapid development of the penicillin industry. Table 4 which gives the highlights in the uses of corn steep as a component of nutrient media should be indicative that the potentialities of this material are beginning to be realized.

In the laboratory, corn steep liquor may serve either as a supplement to replace extracts, or as the main source of nitrogen and carbon for all microörganisms except fastidious pathogens. In general, any organism capable of growing well on simple media containing beef extract and peptone will grow on media containing only corn steep liquor. We have successfully grown the following organisms on one or several of the media mentioned below:

Bacteria	Yeasts and Yeast-like Fungi	Molds
Acetobacter suboxydans	*Candida Guilliermondi*	*Aspergillus flavus*
Bacillus macerans	*Candida lipolytica*	*Aspergillus niger*
Bacillus subtilis	*Endomycopsis fibuliger*	*Aspergillus oryzae*
Lactobacillus arabinosus	*Saccharomyces cerevisiae*	*Eremothecium ashbyii*
Lactobacillus brevis	*Schizosaccharomyces octosporus*	*Mucor boulard*
Lactobacillus casei	*Torulopsis utilis*	*Mucor mucedo*
Lactobacillus delbrueckii	*Zygosaccharomyces japonicus*	*Neurospora crassa*
Lactobacillus fermenti	*Zygosaccharomyces mellis*	*Oospora lactis*
Leuconostoc mesenteroides	*Zygosaccharomyces Nussbaumeri*	*Penicillium notatum*
Micrococcus pyogenes var. aureus	*Zygosaccharomyces Richeri*	*Penicillium chrysogenum*
Pseudomonas aeruginosa		*Phycomyces*
Streptococcus faecalis		*Rhizopus japonicus*
Streptococcus lactis		
Streptomyces cellulosae		
Streptomyces coelicolor		
Streptomyces diastaticus		
Streptomyces flavovirens		
Streptomyces griseus		
Streptomyces microflavus		
Streptomyces olivaceus		

If clear media are desired, preliminary treatment of the crude liquor becomes necessary. A good practice is to adjust the liquor with water until it contains from 15 to 20% solids, raise the pH to 8 with concentrated KOH, autoclave for an hour, and cool and filter. The filtrate then may be reconcentrated or re-

frigerated. For certain purposes, such as penicillin production, the quality of the steep liquor is impaired by this treatment. Refrigeration of corn steep liquor is advisable to prevent spoilage by yeasts.

The following are some representative media. For yeasts, 0.5% corn steep liquor solids, with sugar as desired. For bacteria, 1.0% corn steep liquor solids, 0.5% glucose, adjusted to pH 7.4, as a basal medium. This can be modified in various ways, e.g., for the lactics, 1.0% corn steep liquor solids, 1.5% glucose, in 1.0% phosphate buffer at pH 7.0. Also the following combinations have proved valuable after adjustment to the desired pH: *a*, 0.5% corn steep liquor solids and 0.5% tryptone for bacteria; and *b*, 1.0% corn steep liquor solids and 0.3% yeast extract, for bacteria and yeasts. For media used in industrial processes, the references cited in table 4 should be consulted.

Use in the Production of Penicillin

By far the most important application of corn steep liquor in microbiology was discovered by Moyer and Coghill (44) who noticed that the addition of corn steep liquor to a modified Czapek-Dox solution of mineral salts greatly increased penicillin yields. This discovery encouraged many efforts to isolate *the* active component which endows corn steep liquor with the remarkable ability to stimulate the biosynthesis of penicillin by organisms of the *Penicillium notatum-chrysogenum* group. Since, according to studies on the metabolism of these organisms, the biological function of steep liquor cannot be ascribed to a single factor but to a group of factors (31, 48, 19, 25, 34, 45, 54, 55, 30), such attempts were unsuccessful.

Penicillin appears to be synthesized by the live, active mold and in largest quantities when the pH is between 7.0 and 8.0[4]; therefore, conditions which tend to maintain the mycelium metabolically active and the pH of the medium within the optimum range usually favor maximum penicillin yields. Assuming that certain environmental factors, such as temperature and aeration are kept optimum, these conditions can be provided by a balanced mixture of a variety of nutrients, which includes a readily available source of carbon and nitrogen, a slowly fermentable carbohydrate, and an effective buffer system. The first allows rapid initial formation of the mycelium, the second serves as a slowly available store of energy, which can be tapped throughout the entire period of growth, and the third obviously aids in the maintenance of the pH values within a desired range. Other nutritional factors, such as mineral elements are of course also essential, and will be mentioned later.

Studies of the metabolic changes that occur during the growth of penicillin-producing molds reveal a pattern which is characteristic for good penicillin yields; conditions unfavorable for optimum penicillin production ordinarily find their expression in deviation from the typical metabolic pattern. In this manner it can be demonstrated that corn steep liquor, fortunately enough, is one of the few inexpensive and readily available materials which include a desirable balance

[4] For certain synthetic media the pH best for penicillin production has been found to be 7.3 as compared to 6.8 which seemed optimum for the growth phase of the mold (24).

of as many of the essential factors as possible. Lactic acid is a readily available source of carbon for the penicillin-producing molds; amino acids and polypeptides serve as readily available sources of both carbon and nitrogen and also as buffer systems; the ash of corn steep liquor readily supplies the need of the organism for mineral elements. The only important constituent lacking is a slowly available source of energy, which in the media commonly used in the industry, is furnished as lactose. To obtain maximum penicillin yields the exact composition of corn steep liquor-lactose media has to be varied with the degree of aeration. This depends on whether surface cultures, shake flask fermentations, or large volume tank fermentations are employed (4). The media for large-scale production usually also contain 1.0% $CaCO_3$ to make the reaction of the medium less acid.

The fact that synthetic media can be employed in the production of penicillin supports the view that a combination of factors rather than a single factor accounts for the excellence of steep liquor. For example, glucose and acetic acid may be substituted for the readily available carbon compounds of corn steep liquor, and ammonium salts for the readily available nitrogen compounds. Phosphates serve as buffers; metabolism of acetate controls the pH of certain synthetic media during the primary growth phase, while the pH during the subsequent penicillin-forming phase is greatly influenced by the rate of lactate utilization. Salt solutions containing S, Fe, K, Mg, Zn, Cu, Mn, and Ca in addition to P, may replace the ash fraction of corn steep liquor. Lactose is added as the slowly fermentable carbohydrate. The work on synthetic media done at the Pennsylvania State College (56) and the University of Wisconsin (24) should be consulted for further details.

In addition to the non-specific factors already mentioned, specific precursors of penicillin may occur in steep liquor. Inspection of the structural formulae of the various penicillins (6) suggests a considerable number of compounds which potentially may serve as precursors. However, only a few, such as phenylethylamine and tyramine, which are decarboxylation products of phenylalanine and tyrosine, respectively, have been demonstrated to occur in corn steep liquor (2, 3, 17). Of course, it is now an established industrial practice to stimulate the biosynthesis of penicillin by furnishing precursors of various chemical types to the mold (22, 57, 24, 46, 3).

The early efforts to elucidate the role of corn steep liquor usually were frustrated because they involved adding fractions of steep liquor, or known compounds to basal media from which corn steep liquor had been omitted. Since these basal media ordinarily consisted only of a modified Czapek-Dox solution and lactose, they contained in the absence of corn steep liquor neither enough nitrogen nor phosphorus to permit adequate penicillin production. For example Moyer and Coghill (44) found that neither trace elements nor the redissolved ash of corn steep liquor, nor growth factors, nor amino acids when added to such a basal medium could replace corn steep liquor in its ability to stimulate penicillin production. On the other hand Knight and Frazier (29) showed that the ash of corn steep liquor was stimulatory to penicillin production. These workers

used a basal medium which was adequate in all respects except, as shown later by Koffler, Knight and Frazier (33), in its content of iron and phosphorus. Under these conditions the ash appeared to be *the* important constituent of corn steep liquor because it supplied the needed iron and phosphorus. Similarly, White, Krampitz, and Werkman (67) claimed that a mixture of histidine, arginine, glutamic acid and succinic acid was responsible, at least in part, for the stimulatory activity of corn steep liquor. It would seem now that these acids primarily functioned as readily available sources of carbon and nitrogen in a medium deficient in these factors. All these reports support the thesis that in order to test the essentiality to penicillin production of one nutritional or other factor, all other conditions should be optimal.

The work of Cook and his associates (8, 9, 10, 11, 12, 13, 14, 15, 16) is of interest in this connection. These workers claimed that aqueous extracts of peas, when added to a basal medium, stimulated penicillin yields. After considerable chemical work, which included careful fractionation of the stimulatory extract, they decided that diverse nitrogen and carbon compounds had the same stimulatory effect. Apparently their basal medium was deficient in readily available sources of carbon and nitrogen, and pea extracts influenced the well-being of the molds generally, rather than penicillin synthesis specifically.

The mineral nutrition of the mold also seems to be of great importance to the success of the penicillin fermentation. It is indeed fortunate that corn steep liquor contains so many mineral elements, and therefore adequately supplies the mold with the mineral elements necessary to penicillin formation. Theoretically, mineral elements could serve several functions in penicillin production. Some elements may function *directly* as constituents of enzymes that are essential to mold metabolism in general, or to penicillin synthesis in particular. Or, some mineral elements may act *indirectly* because they protect either the mold from the harmful effects of toxic elements or penicillin from the destruction catalyzed by other elements. Hutner (23) holds the general view that many elements are considered essential nutrients because they form precipitates in dilute media and thereby remove toxic elements by precipitation or adsorption. Of interest are the recent papers by Pulvertaft and Yudkin (53) and Pratt (50, 51, 52) who claim that phosphates specifically stabilize penicillin by effects other than those which they exert as buffers. Pratt suggests phosphorylation of the penicillin molecule. It is also possible that harmful elements are removed as insoluble phosphates. In certain synthetic media, the level of phosphates required to give optimum growth of the mold is lower than the amount to give optimum penicillin yields. This may be explained possibly by the protective effect that phosphates exert on the penicillin. Since at any one time penicillin "yields" are influenced by synthesis and destruction of penicillin (processes that are thought to occur to varying degrees simultaneously), the high content of phosphates in steep liquor may be of additional advantage. Perhaps the increase in penicillin yields, observed in certain cases, when supplements of boric acid or citric acid were added to corn steep liquor-lactose media (32, 44) might be explained similarly. Thomas (61) and Hahn (21) recently have demon-

strated that penicillin is considerably more stable in citrate buffers than in saline or phosphate solutions. It is conceivable that boric acid and citric acid appeared to stimulate penicillin yields because they combine with ions which otherwise would be harmful to the production of penicillin either by poisoning the mold or by catalyzing the destruction of penicillin.

Interestingly enough, better penicillin yields can be obtained from submerged cultures when $CaCO_3$ is used to neutralize the otherwise acid corn steep liquor medium rather than KOH (45) or NaOH (19). Could these higher yields be ascribed to the fact that $CaCO_3$ also adsorbed deleterious materials in addition to raising the pH? In spite of any toxic components, which steep liquor may contain, there is little doubt that it has become a valuable source of nutrients for microörganisms in industry and the laboratory.

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